Amycolatopsis albidoflavus sp. nov.

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The generic position of an actinomycete strain, '*Pseudonocardia* sp.' IMSNU 22139, was investigated by the phylogenetic analysis of 16S rDNA sequence. Comparative studies of 16S rDNA sequences indicated that this organism consistently formed a distinct clade within the radiation of the genus *Amycolatopsis* of the family *Pseudonocardiaceae*. This organism was also found to have chemotaxonomic properties consistent with those of the genus *Amycolatopsis*, which were represented by a type IV cell wall (*meso*-diaminopimelic acid, arabinose and galactose), a major menaquinone of MK-9(H₄), a predominant fatty acid of 14-methylpentadecanoic acid, phosphatidylmethylethanolamine as a diagnostic phospholipid (a phospholipid type PII pattern) and DNA base composition of 68.5 mol% G+C. On the basis of physiological properties, cellular fatty acid profiles and its unique phylogenetic position, this organism is readily differentiated from all of the validly described species of the genus *Amycolatopsis*, and the name *Amycolatopsis albidoflavus* sp. nov. is proposed for it. The type strain is IMSNU 22139^T (= KCTC 9471^T = ATCC 53205^T).

Keywords: *Amycolatopsis albidoflavus* sp. nov., actinomycetes, 16S rDNA sequence studies

INTRODUCTION

The genus *Amycolatopsis* was proposed by Lechevalier *et al.* (1986) for amycolate, nocardioform actinomycetes having a phospholipid type PII pattern (phosphatidylethanolamine as a diagnostic phospholipid) among strains that were previously described as members of the genus *Nocardia*, and currently contains 10 validated species, *Amycolatopsis alba*, *Amycolatopsis azurea*, *Amycolatopsis coloradensis*, *Amycolatopsis fastidiosa*, *Amycolatopsis japonica*, *Amycolatopsis mediterranei*, *Amycolatopsis methanolica*, *Amycolatopsis orientalis* (with *Amycolatopsis orientalis* subsp. *lurida*), *Amycolatopsis sulphurea* and *Amycolatopsis thermoflava* (Lechevalier *et al.*, 1986; Henssen *et al.*, 1987; de Boer *et al.*, 1990; Mertz & Yao, 1993; Labeda, 1995; Goodfellow *et al.*, 1997; Chun *et al.*, 1999).

Members of the genus *Amycolatopsis*, which produce branched, fragmenting aerial and substrate mycelia,

are characterized by the following cell chemistry: a type IV cell wall (*meso*-diaminopimelic acid, arabinose and galactose in the cell wall) (Lechevalier *et al.*, 1986); muramic acid in an *N*-acetyl form (Henssen *et al.*, 1987); phosphatidylethanolamine as a diagnostic phospholipid (a phospholipid type PII pattern according to the classification of Lechevalier *et al.*, 1977); the absence of mycolic acids (Lechevalier *et al.*, 1986); a major menaquinone of MK-9(H₄) with a minor amount of MK-9(H₂) (Lechevalier *et al.*, 1986; Yassin *et al.*, 1993); and complex mixtures of saturated and branched-chain fatty acids (Henssen *et al.*, 1987; Mertz & Yao, 1993). Phylogenetically, this genus forms a monophyletic clade within the radius of the family *Pseudonocardiaceae* (Warwick *et al.*, 1994; Kim & Goodfellow, 1999). '*Amycolatopsis rugosa*', which was originally described as a member of the genus *Amycolatopsis*, was transferred to the new genus *Prauserella* on the basis of 16S rDNA sequence studies and chemotaxonomic characterization (Kim & Goodfellow, 1999).

While investigating the phylogenetic relationship of *Pseudonocardia* strains, we found that '*Pseudonocardia* sp.' IMSNU 22139 was related to members of the genus *Amycolatopsis* (Lee *et al.*, 2000b). In this paper,

The EMBL accession numbers for the 16S rDNA sequences of *Amycolatopsis methanolica* IMSNU 20055^T, *Amycolatopsis azurea* IMSNU 20053^T, *Amycolatopsis fastidiosa* IMSNU 20054^T and *Amycolatopsis orientalis* IMSNU 20058^T reported in this paper are AJ249135, AJ400709, AJ400710 and AJ400711, respectively.

we describe the characterization and classification of '*Pseudonocardia* sp.' IMSNU 22139, for which the new species of the genus *Amycolatopsis*, *Amycolatopsis albidoflavus* sp. nov., is proposed.

METHODS

Micro-organisms and culture conditions. Strain IMSNU 22139 was cultivated on Trypticase Soy Broth (Difco) for 3 d at 30 °C with shaking. After the purity was checked, the cells were harvested by centrifugation and washed twice with distilled water. For phenotypic and genotypic comparison, the following reference strains were used: *Amycolatopsis alba* IFO 15602^T (= IMSNU 22095^T), *Amycolatopsis azurea* IFO 14573^T (= IMSNU 20053^T), *Amycolatopsis coloradensis* IFO 15804^T (= IMSNU 22096^T), *Amycolatopsis fastidiosa* IFO 14105^T (= IMSNU 20054^T), *Amycolatopsis mediterranei* IFO 13142^T (= IMSNU 20056^T), *Amycolatopsis methanolica* IFO 15065^T (= IMSNU 20055^T), *Amycolatopsis orientalis* IFO 12806^T (= IMSNU 20058^T) and *Amycolatopsis sulphurea* IFO 13270^T (= IMSNU 20060^T).

Morphological and physiological characteristics. Acid production from carbohydrates was determined by using Bacto OF basal medium (Difco) including each filter-sterilized compound at a final concentration of 1%. Decomposition of adenine, hypoxanthine, DL-tyrosine and xanthine was examined as described previously (Gordon *et al.*, 1974). Oxidase activity was determined by monitoring the oxidation of tetramethyl-*p*-phenylenediamine on a filter paper disc. Catalase activity was determined with a 3% (v/v) hydrogen peroxide solution. The temperature for growth was tested at 10, 30, 37 and 45 °C. The production of hydrogen sulfide was detected on Trypticase Soy Broth (Difco) by using lead acetate strips. Urease activity was determined by a colour change in Bacto urea broth (Difco). Nitrate reduction, decomposition of hippurate and hydrolysis of casein, gelatin and starch were examined by using previously described methods (MacFaddin, 1980). NaCl tolerance was studied on nutrient agar containing NaCl at final concentrations of 2, 3, 5, 7 and 10% (w/v). For scanning electron microscopy, strain IMSNU 22139 was grown on inorganic salts-starch agar (ISP medium 4) at 28 °C for 14 d. The agar blocks were cut from the growth medium, fixed with 1% osmium tetroxide, and dehydrated through a graded ethanol series, and the ethanol was replaced with isoamyl acetate. After critical point drying, the samples were sputter-coated with gold under a vacuum and observed by a model Stereoscan 260 scanning electron microscope (Cambridge).

Chemotaxonomy. The isomer of diaminopimelic acid was identified using the method of Stanek & Roberts (1974). The sugar compositions of whole-cell walls were determined by GC as described by Saddler *et al.* (1991). The acyl type of the cell wall was analysed by using the method of Uchida & Aida (1984). The presence of mycolic acids was determined by the method of Minnikin *et al.* (1980). Polar lipids were extracted by the small-scale method of Minnikin *et al.* (1984). The purified menaquinones were identified by HPLC (Kroppenstedt, 1985). The phospholipid composition was determined by a previously described method (Minnikin *et al.*, 1977). Cellular fatty acid methyl esters were prepared by a previously described method (Minnikin, 1988) and analysed by GC with a Hewlett Packard model 5890A gas chromatograph as described previously (Lee *et al.*, 2000c).

Determination of DNA base composition. Genomic DNA was extracted according to Hopwood *et al.* (1985). The G+C content of the DNA was determined by HPLC (Mesbah *et al.*, 1989).

Phylogenetic analyses. The PCR cloning and sequencing of 16S rDNA were carried out by using a model ALFexpress DNA sequencer (Pharmacia Biotech) and the Cy5 AutoRead Sequencing Kit (Pharmacia) as described previously (Lee *et al.*, 2000a). Multiple alignment of the sequences determined in this study and reference sequences was performed by using the CLUSTAL W program (Thompson *et al.*, 1994) and was manually optimized by comparison with *Escherichia coli* sequence (Brosius *et al.*, 1978). Phylogenetic analyses were performed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1972) methods. Evolutionary distances for the neighbour-joining method were computed by the method described by Jukes & Cantor (1969). Parsimony analysis was performed with the PAUP program for the Macintosh (Swofford, 1998). The confidence level of tree topologies was evaluated by bootstrap analysis (Felsenstein, 1985) of the neighbour-joining data, using 1000 resamplings. The final tree was constructed using the neighbour-joining method, based on 1307 unambiguous nucleotides present in all strains.

RESULTS AND DISCUSSION

16S rDNA sequence studies showed that '*Pseudonocardia* sp.' IMSNU 22139 was related to members of the genus *Amycolatopsis* of the family *Pseudonocardiaceae* (Lee *et al.*, 2000b). The phylogenetic relationship is also supported by chemotaxonomic properties. Strain IMSNU 22139 was characterized by a type IV cell wall (*meso*-diaminopimelic acid, arabinose and galactose in whole-cell hydrolysates), a major menaquinone of MK-9(H₄), muramic acid in an *N*-acetylated form and a base composition of 68.5 mol%. Mycolic acids were absent. The fatty acid profiles contained a mixture of saturated, unsaturated, and branched-chain acids, iso-hexadecanoic acid being the predominant fatty acid. The polar lipid profiles contained considerable amounts of phosphatidylmethylethanolamine and phosphatidylinositol, along with trace amounts of phosphatidylethanolamine and diphosphatidylglycerol (a phospholipid type PII pattern; Lechevalier *et al.*, 1977).

An almost complete 16S rDNA sequence for the strain IMSNU 22139 obtained in our recent study (Lee *et al.*, 2000b) comprises 1477 nucleotides (> 98% of the *E. coli* sequence). To increase the quality of the reference sequences, almost complete 16S rDNA sequences of the following strains were also determined in this study: *A. azurea* IMSNU 20053^T, *A. fastidiosa* IMSNU 20054^T, *A. methanolica* IMSNU 20055^T and *A. orientalis* IMSNU 20058^T. The newly determined sequences were compared with the corresponding sequences of the remaining validated species of the genus *Amycolatopsis*, and the representatives of the genera *Pseudonocardia* and *Actinobispora*. A total of 1307 unambiguous aligned positions present in all strains between 72 and 1452 (*E. coli* positions) were used for this analysis. *Saccharothrix violacea* (Lee *et*

Fig. 1. Phylogenetic tree displaying the position of '*Pseudonocardia* sp.' IMSNU 22139 with regard to related taxa. Triple asterisks indicate the branching nodes that were also recovered in the parsimony analysis. The numbers at the branching nodes are the percentages of occurrence in 1000 bootstrapped trees (only values greater than 40% were indicated). Scale bar indicates 1 substitution per 100 nucleotides.

al., 2000c) was used as an outgroup. The phylogenetic tree (Fig. 1) shows that strain IMSNU 22139 forms a distinct lineage within the radius of the genus *Amycolatopsis*, which was supported by a high bootstrap value of 98%. Parsimony analysis based on the same data sets resulted in a tree showing similar topologies. The levels of 16S rDNA sequence similarity between strain IMSNU 22139 and members of the validated species of the genus *Amycolatopsis* ranged from 93.7 to 97.4%. Of the members of the genus *Amycolatopsis*, *A. azurea* (97.4%) and *A. orientalis* (97.4%) showed the highest sequence similarity to strain IMSNU 22139. Thus, phylogenetic clustering and chemotaxonomic characterization support strain IMSNU 22139 being a member of the genus *Amycolatopsis*.

The morphological study using a electron microscope showed that strain IMSNU 22139 had a typical characteristic of the genus *Amycolatopsis* (data not shown). Both aerial and vegetative hyphae were branched and fragmented. Other morphological features, such as sclerotia and sporangia, were not observed. All of the test strains including strain IMSNU 22139 produced acid from D-fructose and glycerol, but did not produce acid from D-melezitose, L-rhamnose and L-sorbose. Decomposition of casein, starch and DL-tyrosine was observed in all of the test

strains. Table 1 shows physiological characteristics that differentiate strain IMSNU 22139 and the type species of the genus *Amycolatopsis*. *A. azurea* IMSNU 20053^T in the degradation of starch and acid production from D-raffinose and sucrose, *A. coloradensis* IMSNU 22096^T in H₂S production, *A. fastidiosa* IMSNU 20054^T in the degradation of starch and gelatin, *A. methanolica* IMSNU 20055^T in acid production from L-arabinose, D-galactose, L-rhamnose and D-trehalose and in the decomposition of urea and xanthine, *A. orientalis* IMSNU 20058^T in nitrate reduction and in the degradation of xanthine, and *A. sulphurea* IMSNU 20060^T in decomposition of casein and in acid production from maltose and glycerol showed different results from the previous study (de Boer *et al.*, 1990; Labeda, 1995). On the basis of cellular fatty acid compositions (Table 2), strain IMSNU 22139 differs from the phylogenetic neighbours *A. azurea* and *A. orientalis*. The cell hydrolysates of strain IMSNU 22139 contained 14-methylpentadecanoic acid (i-C_{16:0}; 26.1%) as a predominant fatty acid. Considerable amounts of hexadecanoic acid (C_{16:0}; 10.2%), 12-methyltridecanoic acid (i-C_{14:0}; 9.8%), 13-methyltetradecanoic acid (i-C_{15:0}; 8.7%), heptadecanoic acid (C_{17:0}; 7.8%) and octadecanoic acid (C_{18:0}; 6.2%) were also present in addition to an unknown fatty acid (7.8%). Strain IMSNU 22139 can also be differentiated from the representatives of other

Table 1 Differential characteristics of strain IMSNU 22139 and the type species of the genus *Amycolatopsis*

+, Positive; w, weak positive; —, negative; ND, not determined.

Characteristic	Strain IMSNU 22139	<i>A. alba</i> IMSNU 22095 [†]	<i>A. azurea</i> IMSNU 20053 [†]	<i>A. coloradensis</i> IMSNU 22096 [†]	<i>A. fastidiosa</i> IMSNU 20054 [†]	<i>A. japonica</i> DSM 44213 ^{†*}	<i>A. mediterranei</i> IMSNU 20056 [†]	<i>A. methanolica</i> IMSNU 20055 [†]	<i>A. orientalis</i> IMSNU 20058 [†]	<i>A. sulphurea</i> IMSNU 20060 [†]	<i>A. thermoflava</i> IFO 14333 ^{†*}
Acid produced from:											
Adonitol	+	+	+	—	—	+	—	+	+	—	+
L-Arabinose	+	+	+	—	—	+	+	—	+	—	+
D-Cellobiose	+	+	+	+	—	+	+	+	+	—	+
meso-Erythritol	+	+	+	—	—	+	—	—	+	—	—
D-Galactose	+	+	—	—	—	+	+	—	—	+	+
D-Glucose	—	—	—	—	—	ND	—	w	—	+	ND
meso-Inositol	+	+	+	+	—	+	+	—	+	—	—
D-Lactose	+	+	+	—	—	+	+	—	+	—	+
Maltose	—	+	+	—	—	+	+	—	+	+	—
D-Mannose	+	—	—	—	—	ND	—	+	—	+	ND
D-Mannitol	w	+	+	+	—	+	+	—	+	+	+
Melibiose	—	+	—	—	—	+	+	—	—	—	+
Methyl α -D-glucoside	—	+	+	+	—	+	—	—	+	—	+
D-Raffinose	—	+	+	—	—	+	+	—	—	—	+
Salicin	—	+	+	+	+	+	+	w	+	—	+
D-Sorbitol	—	—	—	—	—	—	—	+	—	—	+
Sucrose	+	—	+	—	+	+	+	—	+	—	—
D-Trehalose	+	+	+	+	—	+	+	—	+	+	+
D-Xylose	+	+	+	+	+	+	+	+	+	—	+
D-Xylitol	—	—	—	—	—	ND	—	+	—	—	ND
Enzyme activity:											
Nitrate reductase	+	—	+	+	+	—	+	+	+	+	+
H ₂ S production	+	+	+	—	—	+	+	+	+	+	ND
Decomposition of:											
Aesculin	+	+	+	+	+	+	+	+	+	—	+
Gelatin	+	+	+	+	+	+	+	—	+	+	—
Hypoxanthine	+	+	+	+	—	+	+	+	+	—	+
Urea	w	+	+	—	—	—	—	+	+	—	+
Xanthine	+	+	+	—	—	+	—	+	+	—	+
Growth at:											
45 °C	—	—	—	—	+	+	+	+	—	—	+
Growth on:											
7% NaCl	+	—	+	—	—	+	w	—	—	—	—

* According to Goodfellow *et al.* (1997) and Chun *et al.* (1999).**Table 2** Cellular fatty acid compositions (%) of strain IMSNU 22139 and the reference strains of the genus *Amycolatopsis*

Fatty acid*	Strain IMSNU 22139 [†]	<i>A. azurea</i> IMSNU 20053 [†]	<i>A. orientalis</i> IMSNU 20058 [†]
12-Methyltridecanoic acid (i-C _{14:0})	9.8		4.3
Tetradecanoic acid (C _{14:0})	1.1		
13-Methyltetradecanoic acid (i-C _{15:0})	8.7	29.9	15.2
12-Methyltetradecanoic acid (ai-C _{15:0})		3.9	1.1
Pentadecenoic acid (C _{15:1})	2.3		1.3
Pentadecanoic acid (C _{15:0})	5.8	6.5	7.2
14-Methylpentadecanoic acid (i-C _{16:0})	26.1	2.9	23.8
Hexadecenoic acid (C _{16:1})	4.0	8.8	3.5
Hexadecanoic acid (C _{16:0})	10.2	5.7	8.6
15-Methylhexadecanoic acid (i-C _{17:0})	1.6	2.6	1.5
14-Methylhexadecanoic acid (ai-C _{17:0})		11.8	13.7
Heptadecenoic acid (C _{17:1})	4.4		1.2
Heptadecanoic acid (C _{17:0})	7.8	25.7	12.6
16-Methylheptadecenoic acid (i-C _{18:1})			1.5
Octadecanoic acid (C _{18:0})	6.2	2.1	3.0

* Values less than 1.0% not shown. i, iso; ai, anteiso.

[†] Also contained unknown fatty acid (7.8%) at the retention time between hexadecanoic acid (C_{16:0}) and 15-methylhexadecanoic acid (i-C_{17:1}).

validated species of the genus *Amycolatopsis* using the combination of fatty acid profiles (Mertz & Yao, 1993; Yassin *et al.*, 1993; Labeda, 1995; Goodfellow *et al.*, 1997; Chun *et al.*, 1999) and its unique phylogenetic position as well as physiological properties.

On the basis of phenotypic and genotypic data, strain IMSNU 22139 merits being recognized as a novel species in the genus *Amycolatopsis*, for which the name *Amycolatopsis albidoflavus* sp. nov. is proposed.

Description of *Amycolatopsis albidoflavus* sp. nov.

Amycolatopsis albidoflavus (al.bi.do fla'vus. L. adj. *albidus* white; L. adj. *flavus* yellow; M.L. adj. *albidoflavus* whitish yellow).

Forms well-developed aerial mycelium and vegetative mycelium. Both of them fragment into rod-shaped elements. The aerial mycelium is white and the vegetative mycelium is yellow. A yellow soluble pigment is produced on rich media. Aerobic, Gram-positive, non-acid-fast. Catalase-positive. H₂S is produced. Nitrate is reduced to nitrite. Growth occurs between 10 and 37 °C. Growth does not occur at 45 °C. Acid is produced from adonitol, L-arabinose, D-cellobiose, D-galactose, *meso*-erythritol, D-fructose, glycerol, *meso*-inositol, D-lactose, D-mannose, sucrose, D-trehalose and D-xylose. No acid is produced from D-glucose, maltose, D-mannitol, D-melezitose, melibiose, methyl α -D-glucoside, D-raffinose, L-rhamnose, salicin, L-sorbose, D-sorbitol and D-xylitol. Casein, aesculin, gelatin, hypoxanthine, starch, DL-tyrosine and xanthine are decomposed. Exhibits weak urease activity. Growth occurs on 7% NaCl. Type IV cell wall (*meso*-diaminopimelic acid, arabinose and galactose in the cell wall). Muramic acid is in an *N*-acetyl form. The major menaquinone is MK-9(H₄). The phospholipid profiles contain phosphatidylmethylethanolamine and phosphatidylinositol (a phospholipid type PII pattern). Mycolic acids are not present. The predominant fatty acid is iso-branched hexadecanoic acid. Saturated, unsaturated and branched-chain fatty acids are also present. The G+C content of the DNA is 68.5 mol%. Isolated from soil. Originally deposited in the ATCC by Bristol-Myers Co. The type strain is IMSNU 22139^T (= KCTC 9471^T = ATCC 53205^T).

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